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Immunophenotypical alterations in a subset of patients with common variable immunodeficiency (CVID)

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SUMMARY

We investigated the expression of surface molecules on lymphocytes from 20 patients with CVID and 40 healthy subjects. Lymphocytes were analysed by dual colour flow cytometry. We identified a subset of patients (8 of 20) characterized by low CD4/CD8 ratio (< 1·1), expansion of T cells co-expressing the activation marker HLA-DR and significant increase in $CD8^+$ T cells co-expressing CD57. Expression of the adhesion molecules LFA-3 (CD58) and ICAM-1 (CD54) was significantly increased in this subgroup. In addition, within the $CD4^+$ T cells the percentage of $CD29^+$ (memory) cells was increased, while the CD45RA and LAM-1 (Leu-8) antigens were depressed. These results indicate that in a subgroup of CVID patients T cells are activated *in vivo* and the $CD57^+CD8^+$ lymphocyte subpopulation, supposed to comprise functional suppressor T cells, is expanded. We suggest a chronic viral infection in these patients, but it is not clear whether this is primary or secondary to the underlying defect.

Keywords lymphocyte surface molecules common variable immunodeficiency T cell subpopulations adhesion molecules

INTRODUCTION

CVID is clinically characterized by hypogammaglobulinaemia, recurrent sinopulmonary infections and gastrointestinal manifestations, such as gardiasis and nodular lymphoid hyperplasia [1]. In addition there is an increased incidence of malignancies, especially of the lymphoreticular system and gastrointestinal tract [2,3]. Some patients with CVID exhibit autoimmune phenomena or sarcoid-like granulomas [4]. In most CVID patients, B cells are normal in number but fail to differentiate into immunglobulin-secreting cells [5–7]. In addition, defective T helper cell function has been described [8] as well as increased T suppressor cell function [9–11]. Autoantibodies against T cells, serum inhibitors or defective interaction between monocytes and T cells [12,13] may also contribute to the development of this disease. To further differentiate immunological and etiological features of CVID we investigated the expression of several lymphocyte surface markers, including adhesion molecules. We tried to correlate immunophenotypical findings with clinical features.

PATIENTS AND METHODS

Patients

The 20 patients studied (seven male, 13 female) were all adults (aged from 28 to 66 years). Clinical characteristics of these patients are presented in Table 1. All patients were receiving regular i.v. gamma globulin replacement at 4–6 week intervals. At the time of evaluation all patients were free of acute infections. Blood samples were taken just before gamma globulin infusion. The results obtained from CVID patients were compared with those of 40 healthy controls (aged 21–57 years).

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Surface-marker phenotyping

Peripheral blood mononuclear cells (PBMC) were isolated from venous EDTA blood by Ficoll–Hypaque density gradient centrifugation. Unfractionated mononuclear cells were incubated with directly fluoresceinated MoAbs for 30 min at 4°C. Stained cells were washed twice and then analysed by two-colour flow cytometry in a FACStar (Becton Dickinson).

Monoclonal antibodies

Pan T cell markers used were anti-CD2 (IOT 11, Dianova), anti-CD3 (Leu-4, Becton Dickinson) and anti-CD5 (Leu-1, Becton Dickinson). Monoclonal reagents for the following subset markers were employed: CD4 (Leu-3a, Becton Dickinson), CD8 (Leu-2a, Becton Dickinson), CD29 (4B4, Coulter), CD45RA (2H4, Coulter), anti-HLA-DR (Becton Dickinson), CD25 (IOT 14, Dianova), TCR-$\alpha\beta$ (WT31, Becton Dickinson) and CD57 (Leu-7, Becton Dickinson). Natural killer (NK) cell specific markers examined were CD16 (Leu-11a, Becton Dickinson) and CD57 (Leu-7, Becton Dickinson). B cell markers used were anti-

Table 1. Selected clinical features of patients with CVID

No.	Age	Sex	Serum-immunoglobulin (g/l)* IgG	IgM	IgA	Splenomegaly	Autoimmune disorders†	Skin test‡	NLH§
1	30	M	3·6	0·4	0·9	–	–	Path.	?
2	37	M	2·9	0·2	0·4	+	–	Norm.	–
3	31	F	1·8	0·3	0·3	–	–	NT	?
4	34	F	1·8	0·3	0·3	+	–	Path.	–
5	54	F	7·8	0·2	0·3	–	+	NT	?
6	28	F	6·7	1·3	0·4	–	+	Path.	+
7	64	F	4·8	0·3	0·3	+	–	Path.	–
8	30	M	4·0	0·4	0·3	++	+	Path.	+
9	47	F	2·9	0·6	0·3	–	–	Path.	+
10	65	F	3·8	0·4	1·1	–	–	Path.	–
11	31	F	4·5	0·3	0·3	+	–	Norm.	–
12	66	F	2·6	0·2	0·3	–	+	Path.	+
13	35	M	3·3	0·3	0·3	–	–	Path.	+
14	36	M	4·2	2·9	2·7	–	–	Path.	+
15	41	F	3·6	13·7	1·7	+	–	Path.	–
16	51	F	6·6	0·2	0·3	–	–	Norm.	+
17	29	F	3·9	0·2	0·3	–	–	Path.	–
18	30	M	2·8	0·4	0·4	+	+	Norm.	–
19	45	M	2·7	0·2	0·3	+	–	Norm.	?
20	55	F	2·2	0·2	0·3	+	–	Path.	+

* Normal ranges: IgG, 8–18; IgM, 0·6–2·8; IgA, 0·5–4·5.

† Presence of autoimmune disorders such as pernicious anaemia, haemolytic anaemia and thrombocytopenia.

‡ Merieux Multitest skin reaction was considered pathologically reduced if less than three test antigens induced a significant reaction and the sum of the mean diameters was less than 10 mm.

§ Nodular lymphoid hyperplasia.

NT, Not tested.

CD20 (Leu-16, Becton Dickinson) and anti-surface immunglobulin (sIg; fluoresceinated $F(ab)_2$-goat anti-human IgG, IgA, IgM, Ortho). Monoclonals identifying adhesion molecules were specific for CD11a (LFA-1α, IOT 16, Dianova), CD18 (LFA-1β, IOT 18, Dianova), CD54 (ICAM-1, Dianova), CD58 (LFA-3, TS 2.9 and PAK 1, gift from Prof. S. C. Meuer, Heidelberg, Germany) and LAM-1 (Leu-8, Becton Dickinson). The presence of monocytes was excluded by gating for lymphocytes and using CD14 (My 4, Coulter) as gate control marker.

All antibodies except those specific for adhesion molecules (CD11a, CD18, CD54 and CD58) were directly conjugated with either FITC or PE. The adhesion molecules were indirectly stained using first the unlabelled murine MoAbs and then FITC-labelled goat anti-mouse $F(ab')_2$ fragments (Dianova). One- and two-colour fluorescence assays performed are shown in Table 2.

Statistical analysis

Statistical analysis of the per cent positive cells for each surface molecule was performed using the Mann–Whitney *U*-test. For some selected pairs of surface molecules Pearson's and Spearman's correlation coefficients were calculated.

RESULTS

Identification of a CVID subset

Enumeration of lymphocyte surface molecules revealed that some patients (8/20) clearly differed from healthy controls. The main feature that distinguished the patients was a reduced CD4/

Table 2. Monoclonal reagents used in fluorescence assays

Marker PE labelled	Marker FITC labelled	Significance
Two-colour fluorescence assays		
IgG2	IgG1	Negative control
CD2	CD14	Gate control
CD8	CD4	CD4/CD8 ratio
CD45R4A	CD4	Naive $CD4^+$ T cells
CD29	CD4	Memory $CD4^+$ T cells
LAM-1	CD4	LAM-1^+ $CD4^+$ T cells
CD3	HLA-DR	Activated T cells
CD3	CD25	IL-2R expressing T cells
CD3	TCR-α/β	TCR-α/β expressing T cells
CD20	CD25	IL-2R expressing B cells
CD20	CD5	Autoreactive B cells
CD8	CD16	NK cell subset
CD8	CD57	Suppressor/cytotoxic T cells
CD3	CD16	NK cell subset
One-colour fluorescence assays		
CD11a, CD18, CD54, CD58, sIg		

NK, Natural killer; sIg, surface immunoglobulin.

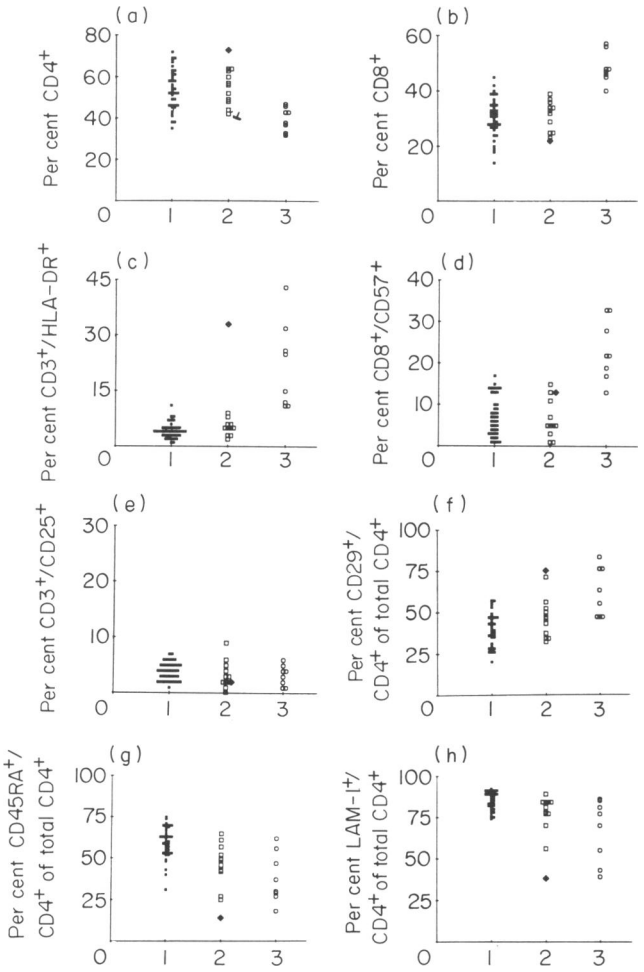

Fig. 1. T lymphocyte subpopulations: 1, controls; 2, CVID patients with CD4/CD8 ratio > 1·1; 3, CVID patients with CD4/CD8 ratio < 1·1. The expression of surface molecules is shown as percentage of lymphocytes, except for f, g and h, where it is shown as percentage of $CD4^+$ T cells. (a) Per cent $CD4^+$ lymphocytes. (b) Per cent $CD8^+$ lymphocytes. (c) Per cent $CD3^+$ and HLA-DR^+ lymphocytes. (d) Per cent $CD8^+$ and $CD57^+$ lymphocytes. (e) Per cent $CD3^+$ and $CD25^+$ lymphocytes. (f) Per cent $CD29^+$ and $CD4^+$ of total $CD4^+$ lymphocytes. (g) Per cent $CD4^+$ and $CD45RA^+$ of total $CD4^+$ lymphocytes. (h) Per cent LAM-1^+ and $CD4^+$ of total $CD4^+$ lymphocytes.

CD8 ratio (< 1·1). Both the decrease in $CD4^+$ lymphocytes and the increase in $CD8^+$ T cells accounted for the significant decrease in the CD4/CD8 ratio (Fig. 1a, b).

One patient was not suitable for this classification. He had an exceptionally high CD4/CD8 ratio (3·3), but other results obtained were consistent with the group of low CD4/CD8 ratio. Clinically this patient was the only one belonging to a CVID subgroup with sarcoid-like granulomas in liver and bone marrow. This patient is identified in the figures.

The most striking features of patients with a low CD4/CD8 ratio were the enhancement of the activation marker HLA-DR on T cells and the increase in $CD8^+$ cells coexpressing CD57 (Fig. 1c, d). In contrast to HLA-DR, CVID patients showed no significant difference in expression of IL-2 receptors (CD25) (Fig. 1e). The enhancement of CD25 expression is said to be one of the first events after T cell activation.

Within the $CD4^+$ T cells the proportion of $CD29^+$ (memory) T cells was elevated (Fig. 1f), whereas the expression of CD45RA (naive) and LAM-1 (Leu-8 antigen) was diminished (Fig. 1g, h). In addition, patients with a low CD4/CD8 ratio exhibited an increased expression of the adhesion molecules LFA-3 (CD58) and ICAM-1 (CD54) on their lymphocytes (Fig. 2a, b). There was a strong statistical correlation between the expression of LFA-3 and ICAM-1. In addition, the expression of these two molecules was also correlated with the expression of the memory T cell marker CD29 (Fig. 2c). These data are consistent with previously reported work that demonstrates the enhancement of LFA-3 and ICAM-1 on memory T cells [14,15].

There was no correlation between the immunophenotypical alterations and clinical features (such as sinopulmonary infections, gastrointestinal manifestations, splenomegaly, autoimmune phenomena or Merieux Multitest skin reaction), nor to a classification of CVID patients on the basis of *in vitro* immunoglobulin synthesis previously reported [16] (results not shown).

Characteristics of all CVID patients studied

T cells. In all patients there was a slight increase in $CD3^+$ cells. These $CD3^+$ lymphocytes were mainly T cells expressing the α/β T cell receptor.

NK cells. The percentage of NK cells ($CD16^+CD3^-$) was significantly reduced ($P < 0.001$). The $CD16^+$ subset coexpressing CD8 (low density) was also significantly reduced ($P < 0.001$). $CD16^+CD3^+$ cells which normally represent less than 1–2% of peripheral blood lymphocytes (PBL) were not altered in CVID patients.

B cells. To assess B cell numbers we evaluated the expression of CD20 and sIg on lymphocytes. There was no decrease, but rather a slight increase of B cell counts in some CVID patients (Fig. 3a, b). No differences were observed in the expression of IL-2 receptors (CD25) or CD5 antigen on B cells as compared with healthy controls (Fig. 3c, d).

Adhesion molecules. Compared with healthy controls, all patients normally expressed LFA-1 (both α- and β-chain) and CD2 on their lymphocytes. On monocytes, CVID patients did not differ from healthy controls in any of the assessed adhesion molecules (LFA-1 α/β-chain, LFA-3 and ICAM-1). In particular there was no decrease or deletion of these surface molecules.

DISCUSSION

Although functional B cell abnormalities have been reported in patients with CVID [5–7,17], previous studies provide evidence for a variety of defects in other cell types. In particular, imbalances of regulatory T cells have been observed in many cases [8,11]. Alterations on monocytes may also contribute to disturbed interactions between lymphocyte subsets [12,13].

All patients studied exhibited normal to elevated numbers of circulating B cells. In addition there was no evidence for a defective or reduced expression of adhesion molecules on monocytes. However, eight out of 20 CVID patients showed significant alterations in their T cell subpopulations. One characteristic finding in these patients was a significantly reduced CD4/CD8 ratio, resulting from both an increase in $CD8^+$ and a decrease in $CD4^+$ T cells. A subset of CVID patients with a reduced CD4/CD8 ratio and other immunophe-

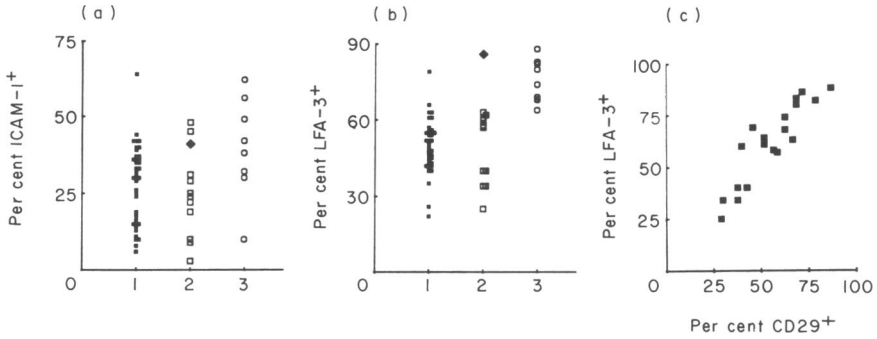

Fig. 2. Expression of adhesion molecules on lymphocytes. 1, Controls; 2, CVID patients with CD4/CD8 ratio > 1·1; 3, CVID patients with CD4/CD8 ratio < 1·1. (a) Per cent ICAM-1$^+$ lymphocytes. (b) Per cent LFA-3$^+$ lymphocytes. (c) Correlation between the expression of LFA-3 and CD29 on lymphocytes.

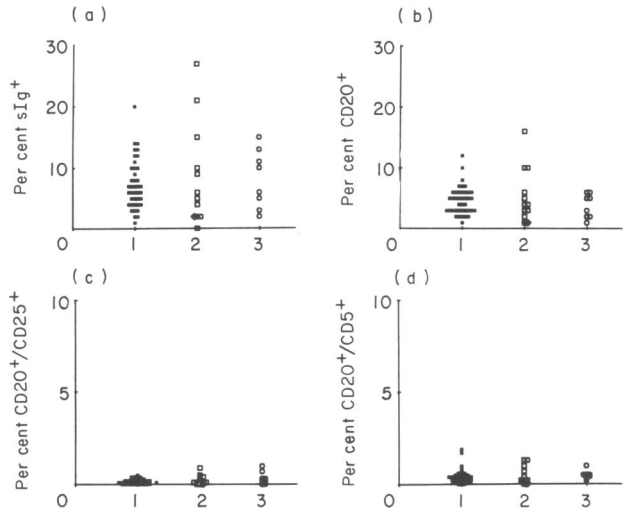

Fig. 3. B lymphocyte subpopulations. 1, Controls; 2, CVID patients with CD4/CD8 ratio > 1·1; 3, CVID patients with CD4/CD8 ratio < 1·1. (a) Per cent surface immunglobulin (sIg) expressing lymphocytes. (b) Per cent CD20$^+$ lymphocytes. (c) Per cent CD20$^+$ and CD25$^+$ lymphocytes. (d) Per cent CD20$^+$ and CD5$^+$ lymphocytes.

notypic and clinical features was first reported by Wright *et al.* [18].

Within the CD4$^+$ T cells the expression of CD45RA was diminished. In contrast, the proportion of CD4$^+$ T cells coexpressing CD29 was elevated. CD45RA$^+$ T cells are known to represent naive T cells, while CD29 is a marker of memory (previously activated) T cells [19–22]. Memory T cells respond much better than naive T cells to recall antigens [23] and they express increased levels of other surface molecules such as the adhesion molecules CD2, LFA-1, LFA-3 (CD58) and ICAM-1 (CD54) [14,15,24]. These adhesion molecules are known to be functionally important in the enhanced responsiveness of memory T cells [25]. In CVID patients with a low CD4/CD8 ratio, the percentage of lymphocytes expressing the adhesion molecules LFA-3 and ICAM-1 was expanded. In addition, there was a strong statistical correlation between the expression of LFA-3, ICAM-1 and CD29. These results indicate that the proportion of memory T cells is elevated in these patients.

During the process of T cell activation there are early and late changes in expression of surface molecules. Whereas IL-2 receptors and HLA-DR appear early after stimulation, the enhancement of CD29 and other adhesion molecules are late events. In contrast to the memory markers CD29, LFA-3, etc., early surface molecules are lost some time after T cell activation. The enhancement of the activation marker HLA-DR on T cells was one of the most striking features of patients with a reduced CD4/CD8 ratio. However, T cells from these patients failed to display increased percentages of IL-2 receptors. In this respect, there have been contradictory reports in other studies of CVID patients: Malkovsky *et al.* [26] found a decrease in IL-2 receptors, while Raziuddin *et al.* [27] noted an enhancement of this activation marker.

Additional evidence for T cell activation was demonstrated by the decline in LAM-1 (Leu-8) expression on CD4$^+$ lymphocytes. LAM-1 has been reported to be lost on activation of T cells [28]. It may have functional importance as a lymphocyte recirculation receptor, but these functional properties have not yet been adequately investigated. It is not clear why the obvious T cell activation in CVID patients with a reduced CD4/CD8 ratio did not result in an increased expression of IL-2 receptors. Interestingly, similar observations have been made in HIV-infected patients [29,30]. Furthermore, a segregation of both the T cell activation markers, HLA-DR and IL-2 receptor, has been described in rheumatoid arthritis [31].

One possibility might be a selective T cell activation defect, as suggested by Sneller & Strober [32]. They noted that T cells from CVID patients showed a significant decrease in mitogen-induced lymphokine gene expression of IL-2, IL-4, IL-5 and interferon-gamma (IFN-γ). Additionally, there are reports of decreased T cell proliferation and IL-2 production *in vitro* [13,16,33,34], as well as decreased IL-4 and IFN-γ production [35] in some patients with CVID. In some cases these abnormalities were correctable *in vitro* by the addition of purified IL-2 or phorbol myristate acetate (PMA). A deficient IL-2 production during T cell activation may account for a reduced IL-2 receptor expression, since IL-2 up-regulates its own receptor [36]. Another possible explanation for the failure of IL-2 receptor expression is that CD25 (IL-2 receptor) and HLA-DR may be expressed during different stages of T cell activation.

Patients with a low CD4/CD8 ratio also exhibited increased levels of CD8$^+$ T cells coexpressing CD57, a lymphocyte subpopulation often found in viral infections, for example during cytomegalovirus (CMV) [37–39] or HIV infections [40–42]. CD8$^+$CD57$^+$ lymphocytes are able to suppress both T cell proliferation and B cell differentiation *in vitro* [43,44]. Phillips &

Lanier [45] reported that lectin-dependent and anti-CD3 induced cytotoxicity are mediated by T lymphocytes expressing CD57. They also demonstrated that more than 80% of these cells were $CD8^+$ and 30% $HLA\text{-}DR^+$, but the cells failed to express CD25 and CD16. Phillips & Lanier proposed that these cells are *in vivo* primed CTL, possibly against virus-infected target cells. Their results were confirmed by Rüthlein *et al.* [46] who reported that $CD8^+CD57^+$ T cells may also suppress immunglobulin synthesis *in vitro*. Taken together, these data provide evidence that $CD8^+CD57^+$ lymphocytes represent a functionally heterogeneous subpopulation and that a chronic viral infection may well explain the enhancement of such cells *in vivo*. In this context it is of interest that hypogammaglobulinaemia has been described following viral infections, such as CMV pneumonia [47]. Döcke *et al.* [48] observed a chronic CMV infection in most CVID patients they studied. Following Epstein–Barr virus (EBV)-infection activation of suppressor T cells may lead to inhibition of B cell activation [49]. An X-linked familial defect of the surveillance mechanisms of EBV infection has been reported by several others [50,51]. It may also cause a fatal lymphoproliferative syndrome (XLPS), aplastic anaemia, agranulocytosis and hypogammaglobulinaemia.

We suggest that the T cell activation and the enhancement of memory T cells in a subgroup of CVID patients may result from a chronic viral infection. Whether such an infection is primary or secondary to the underlying immunodeficiency is unclear. However, other etiological factors may be involved, particularly genes on chromosome 6 which have been linked to CVID [52].

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